Figure 1

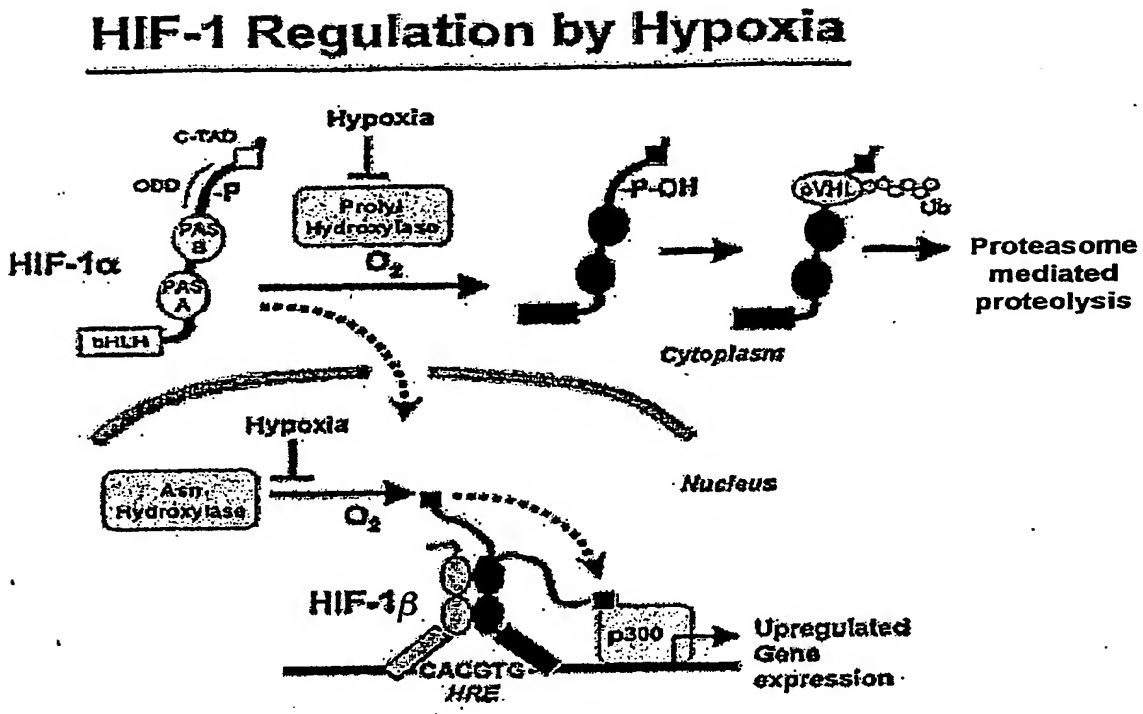

HIF-1 Regulation by Hypoxia
Hypoxia
C-TAD
ODD
-P
PAS B
PAS A
bHLH
HIF-1α
Prolyl Hydroxylase
O_2
-P-OH
pVHL
Ub
Proteasome mediated proteolysis
Cytoplasm
Hypoxia
Asn Hydroxylase
O_2
Nucleus
HIF-1β
p300
Upregulated Gene expression
CACGTG
HRE

Figure 2

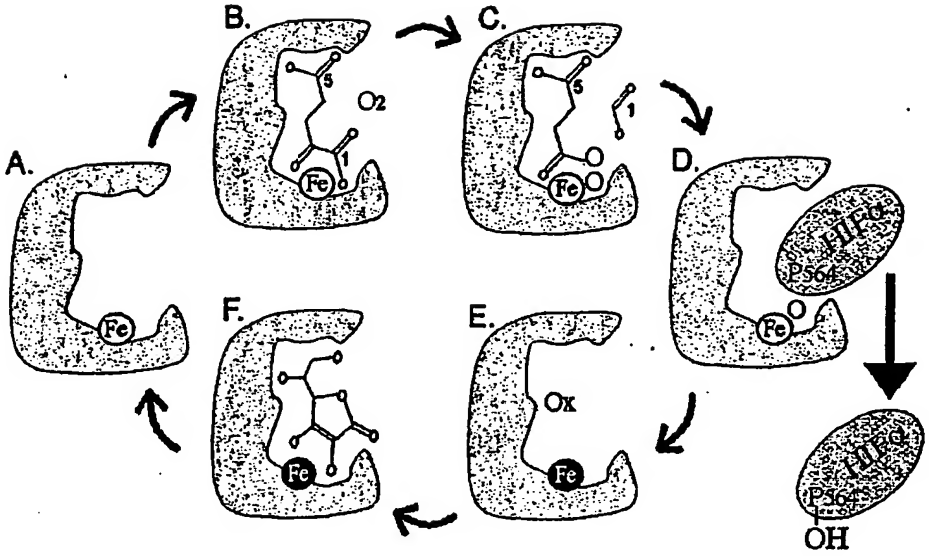
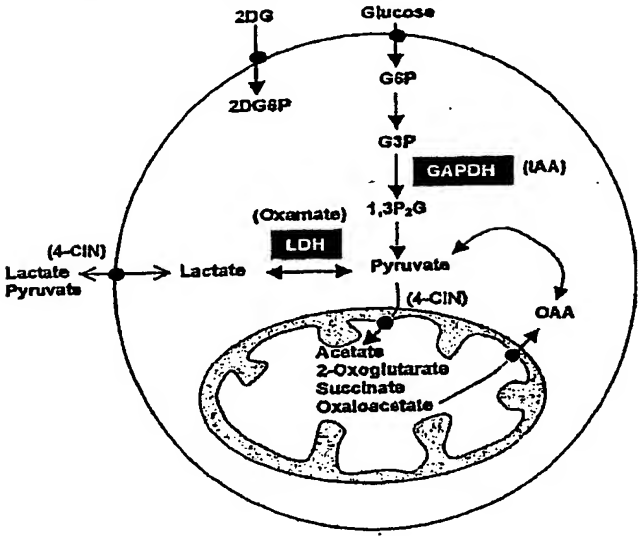


Figure 3

A. Abbreviated diagram of oxygen-independent glucose metabolism and relevant inhibitors.



B. Structural comparisons of key glucose metabolites.

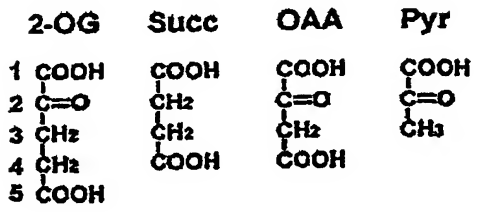


Figure 4

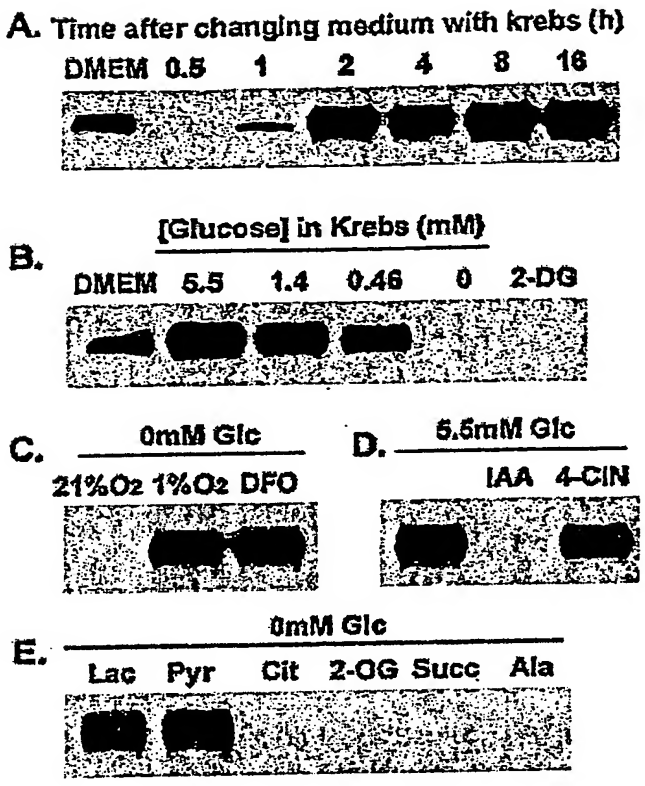

A. Time after changing medium with krebs (h)
DMEM 0.5 1 2 4 8 16
B.
[Glucose] in Krebs (mM)
DMEM 5.5 1.4 0.46 0 2-DG
C.
0mM Glc
21%O2 1%O2 DFO
D.
5.5mM Glc
IAA 4-CIN
E.
0mM Glc
Lac Pyr Cit 2-OG Succ Ala

Figure 5

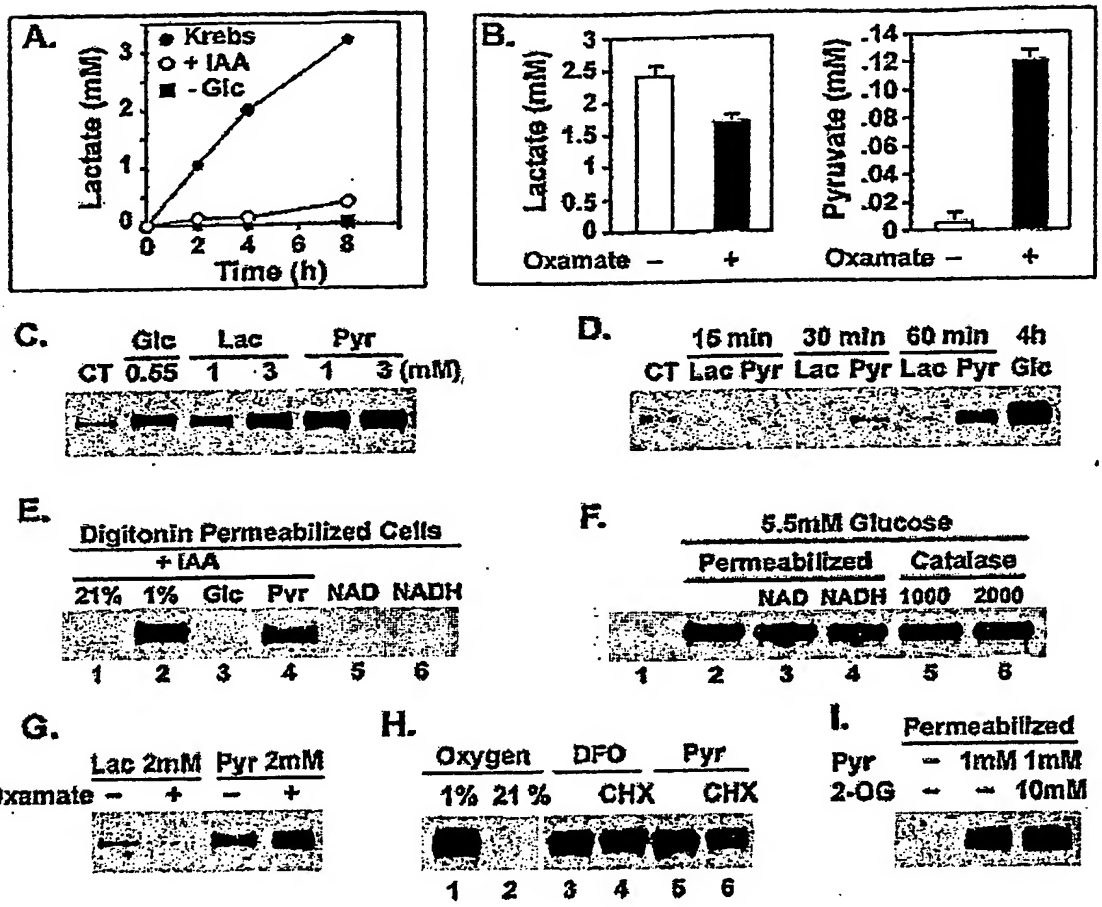

A.
Krebs
+ IAA
- Glc
Lactate (mM)
Time (h)
B.
Lactate (mM)
Pyruvate (mM)
Oxamate – +
Oxamate – +
C.
Glc Lac Pyr
CT 0.55 1 3 1 3 (mM)
D.
15 min 30 min 60 min 4h
CT Lac Pyr Lac Pyr Lac Pyr Glc
E.
Digitonin Permeabilized Cells
+ IAA
21% 1% Glc Pyr NAD NADH
1 2 3 4 5 6
F.
5.5mM Glucose
Permeabilized Catalase
NAD NADH 1000 2000
1 2 3 4 5 6
G.
Lac 2mM Pyr 2mM
Oxamate – + – +
H.
Oxygen DFO Pyr
1% 21 % CHX CHX
1 2 3 4 5 6
I.
Permeabilized
Pyr – 1mM 1mM
2-OG – – 10mM

Figure 6

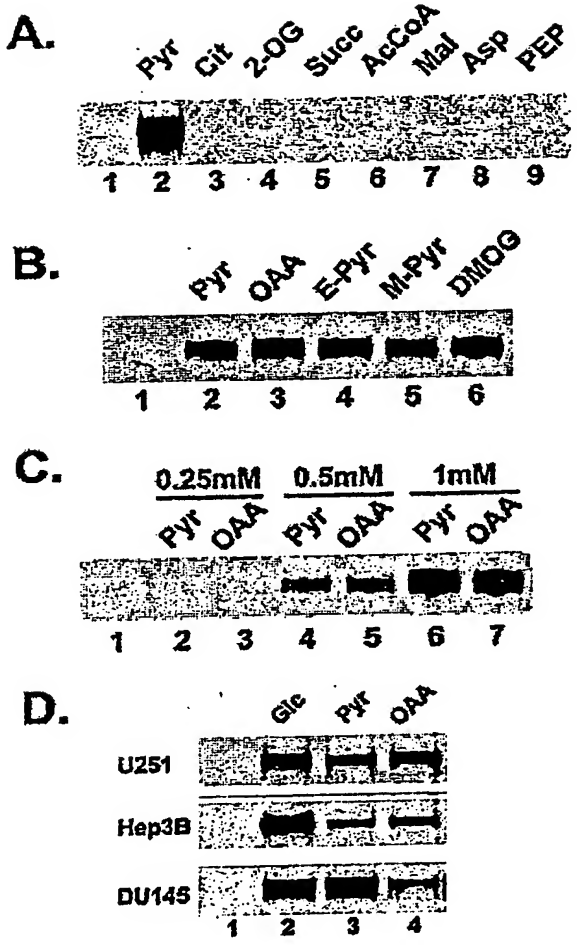

A.
Pyr
Cit
2-OG
Succ
AcCoA
Mal
Asp
PEP
1 2 3 4 5 6 7 8 9
B.
Pyr
OAA
E-Pyr
M-Pyr
DMOG
1 2 3 4 5 6
C.
0.25mM
0.5mM
1mM
Pyr
OAA
Pyr
OAA
Pyr
OAA
1 2 3 4 5 6 7
D.
Glc
Pyr
OAA
U251
Hep3B
DU145
1 2 3 4

Figure 7

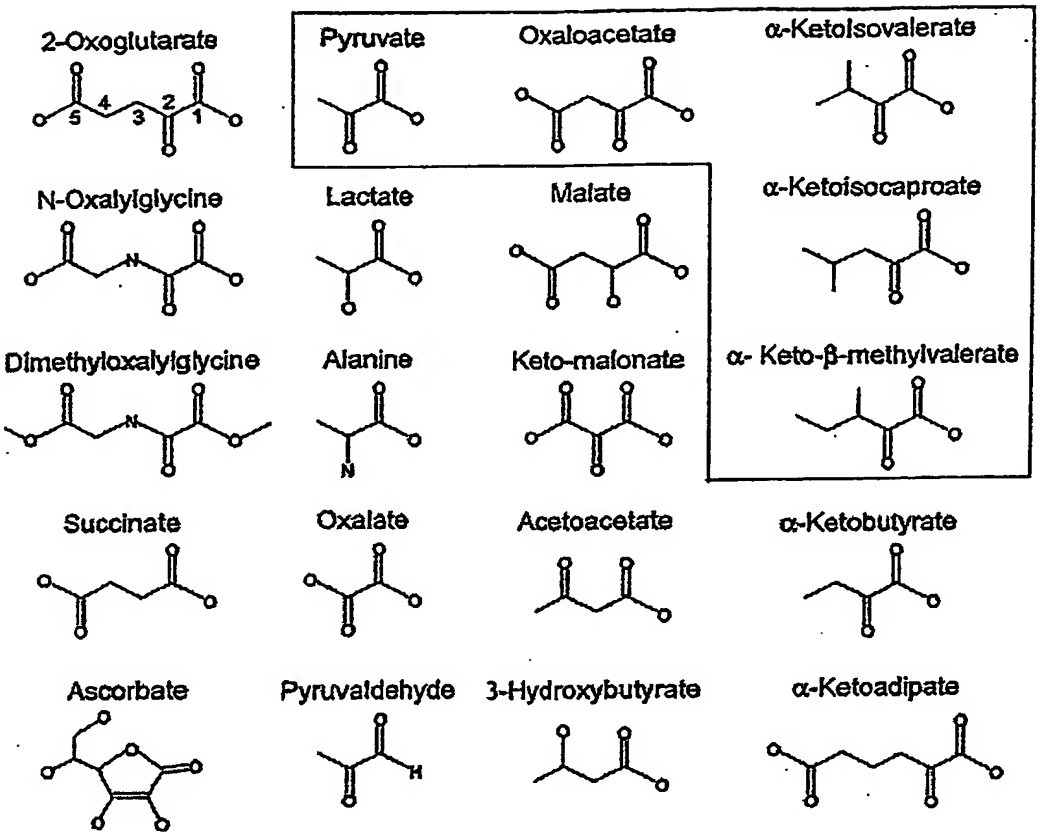

2-Oxoglutarate
Pyruvate
Oxaloacetate
α-Ketoisovalerate
N-Oxalylglycine
Lactate
Malate
α-Ketoisocaproate
Dimethyloxalylglycine
Alanine
Keto-malonate
α- Keto-β-methylvalerate
Succinate
Oxalate
Acetoacetate
α-Ketobutyrate
Ascorbate
Pyruvaldehyde
3-Hydroxybutyrate
α-Ketoadipate

Figure 8

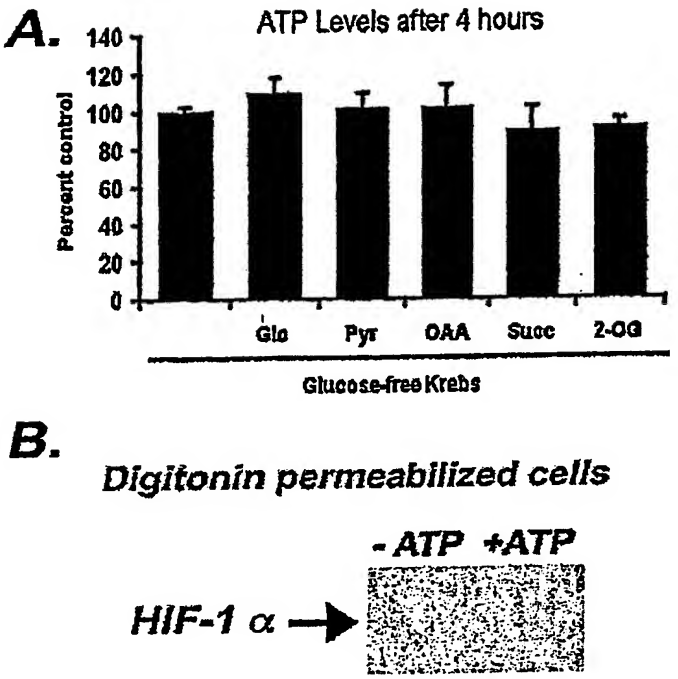

A.
ATP Levels after 4 hours
Percent control
140
120
100
80
60
40
20
0
Glc
Pyr
OAA
Succ
2-OG
Glucose-free Krebs
B.
Digitonin permeabilized cells
- ATP +ATP
HIF-1 α

Figure 9

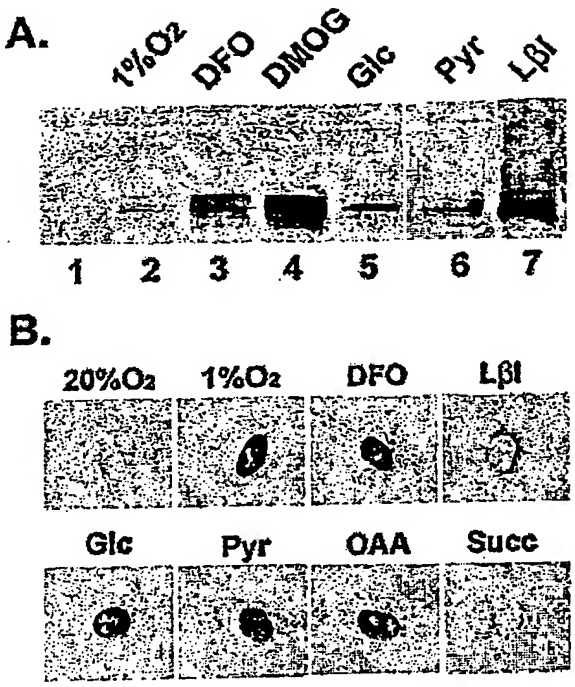

A.
1%O2
DFO
DMOG
Glc
Pyr
Lβl
1 2 3 4 5 6 7
B.
20%O2
1%O2
DFO
Lβl
Glc
Pyr
OAA
Succ

Figure 10

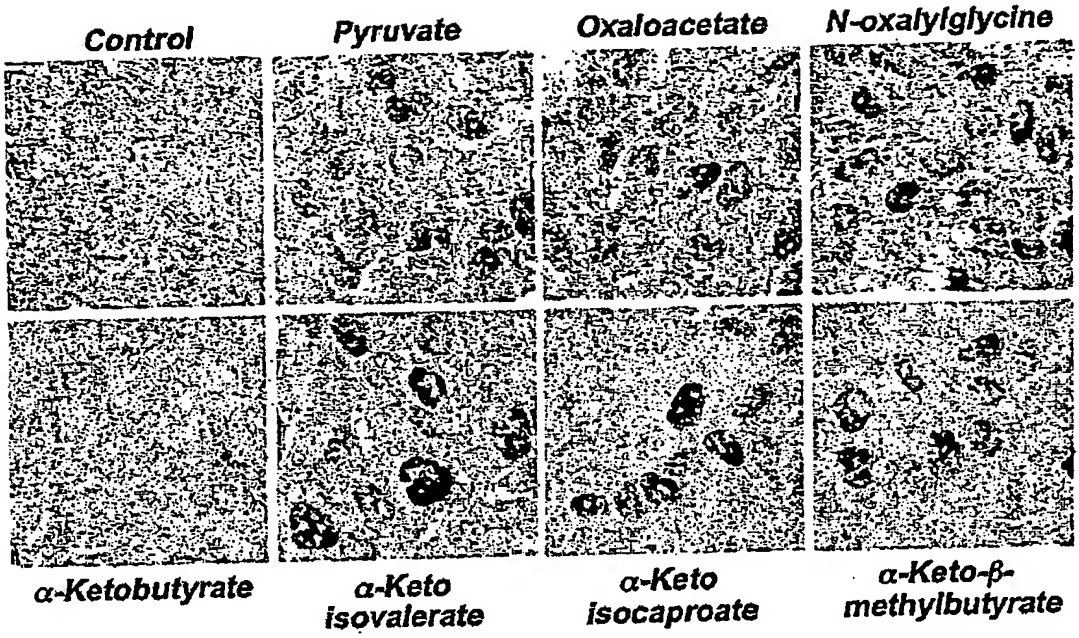

Control
Pyruvate
Oxaloacetate
N-oxalylglycine
α-Ketobutyrate
α-Keto isovalerate
α-Keto isocaproate
α-Keto-β-methylbutyrate

Figure 11

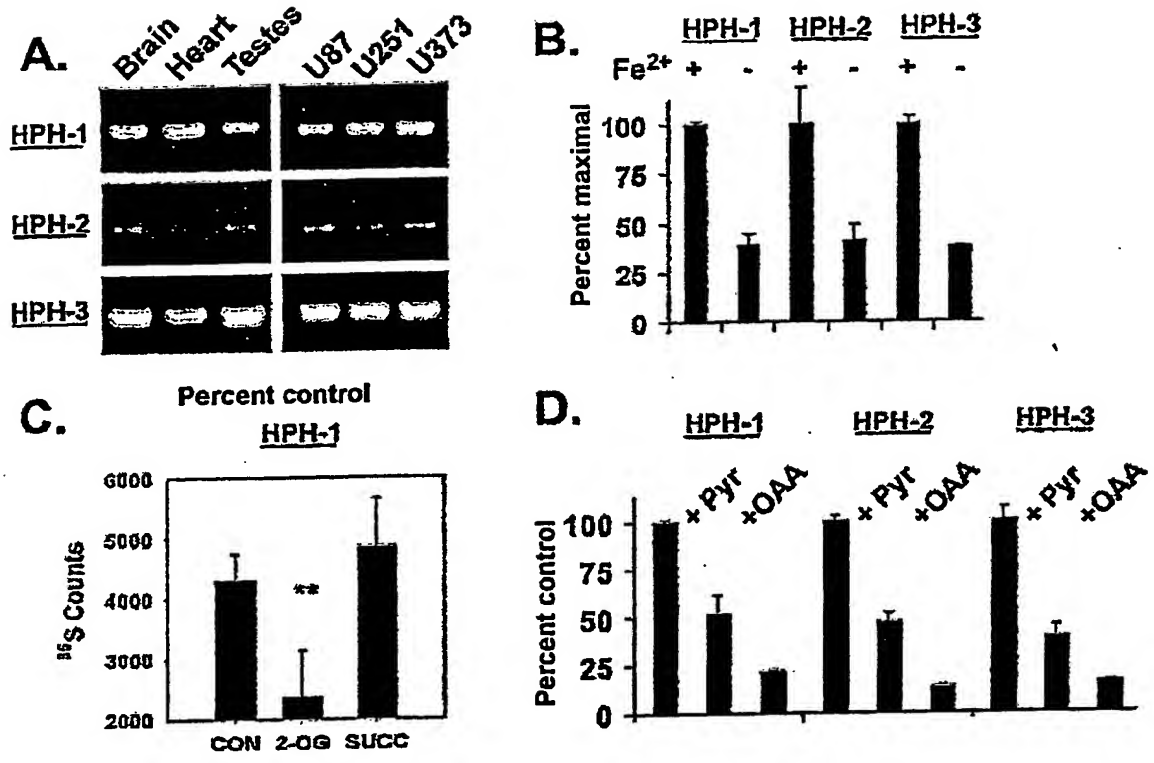

A.
Brain
Heart
Testes
U87
U251
U373
HPH-1
HPH-2
HPH-3
B.
HPH-1
HPH-2
HPH-3
Fe2+ + - + - + -
Percent maximal
100
75
50
25
0
C.
Percent control
HPH-1
6000
5000
4000
3000
2000
35S Counts
**
CON 2-OG SUCC
D.
HPH-1
HPH-2
HPH-3
+Pyr
+OAA
Percent control
100
75
50
25
0

Figure 12

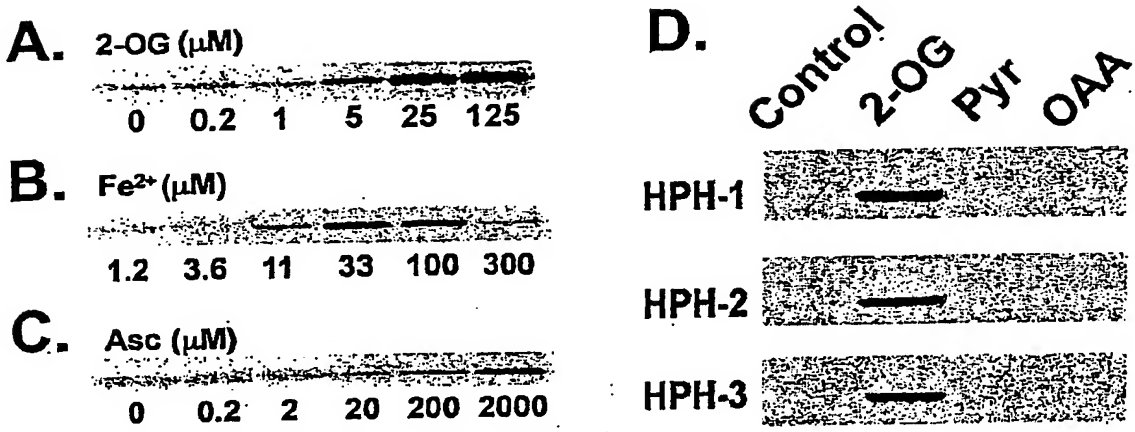

A.
2-OG (μM)
0 0.2 1 5 25 125
B.
Fe2+ (μM)
1.2 3.6 11 33 100 300
C.
Asc (μM)
0 0.2 2 20 200 2000
D.
Control
2-OG
Pyr
OAA
HPH-1
HPH-2
HPH-3

Figure 13

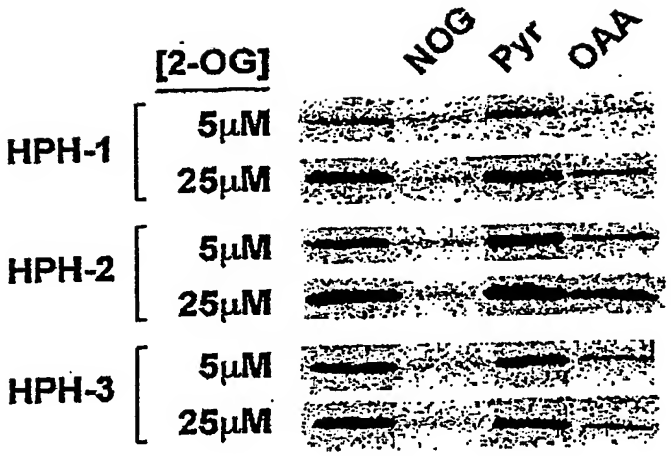


Figure 14

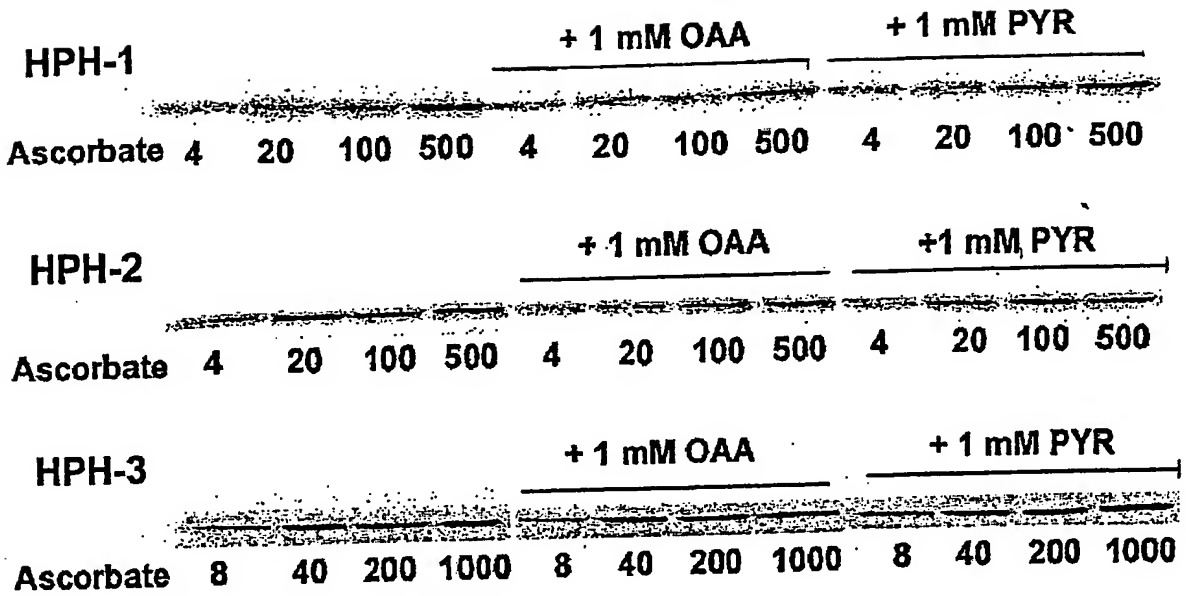

HPH-1
+ 1 mM OAA
+ 1 mM PYR
Ascorbate 4 20 100 500 4 20 100 500 4 20 100 500
HPH-2
+ 1 mM OAA
+1 mM PYR
Ascorbate 4 20 100 500 4 20 100 500 4 20 100 500
HPH-3
+ 1 mM OAA
+ 1 mM PYR
Ascorbate 8 40 200 1000 8 40 200 1000 8 40 200 1000

Figure 15

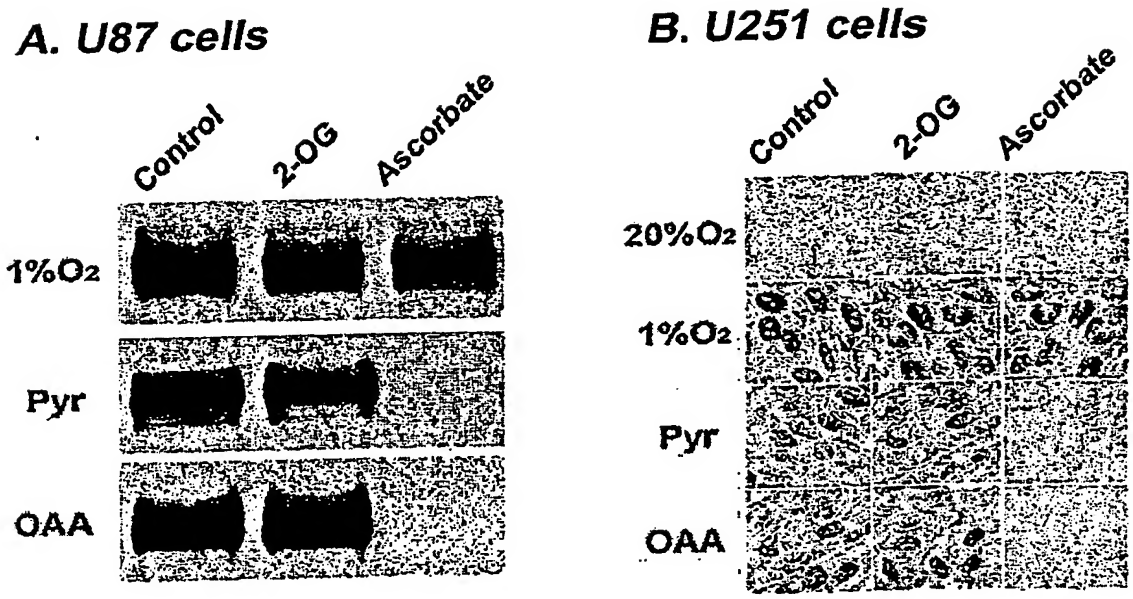

A. U87 cells
Control
2-OG
Ascorbate
1%O2
Pyr
OAA
B. U251 cells
Control
2-OG
Ascorbate
20%O2
1%O2
Pyr
OAA

Figure 16

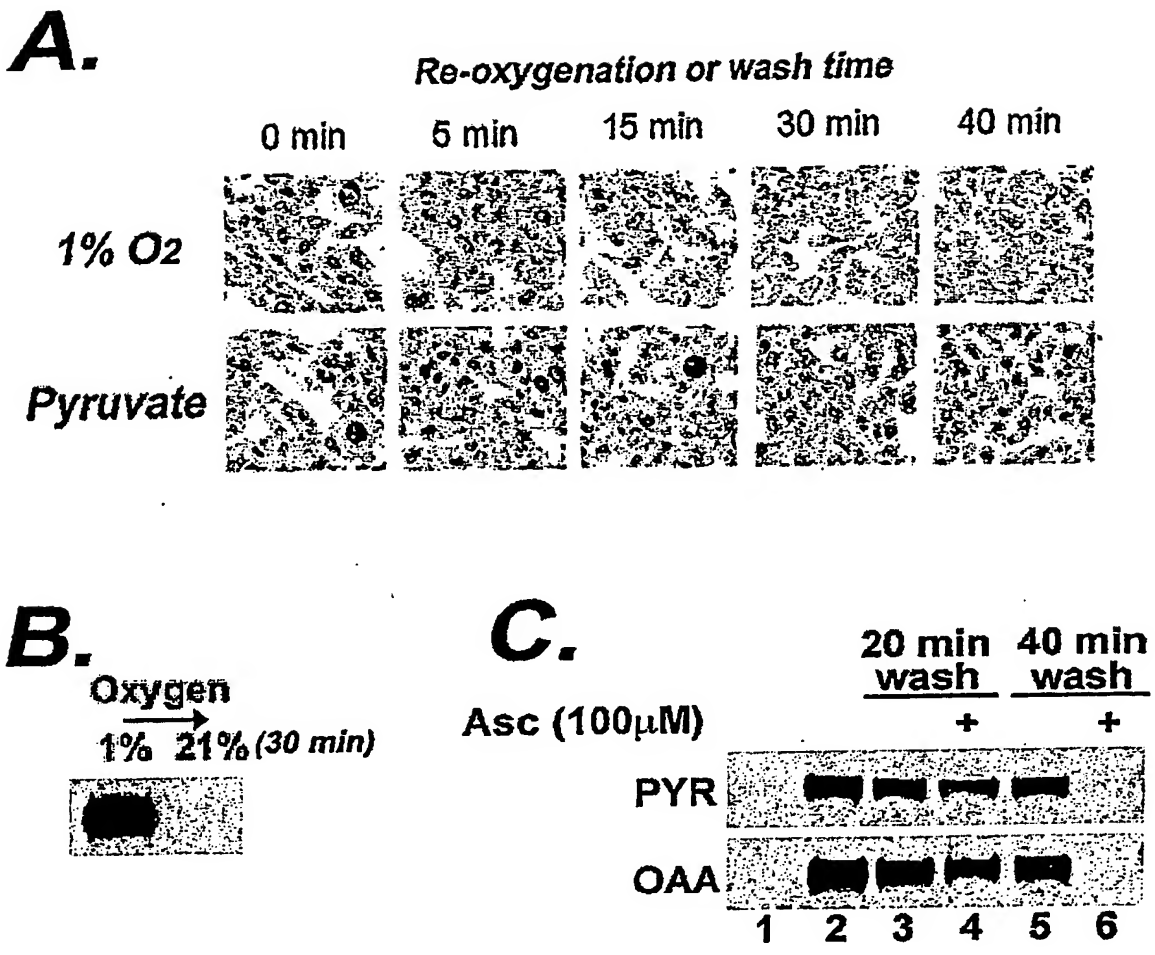

A.
Re-oxygenation or wash time
0 min
5 min
15 min
30 min
40 min
1% O2
Pyruvate
B.
Oxygen
1% 21% (30 min)
C.
20 min wash
40 min wash
Asc (100μM)
+
+
PYR
OAA
1 2 3 4 5 6

Figure 17

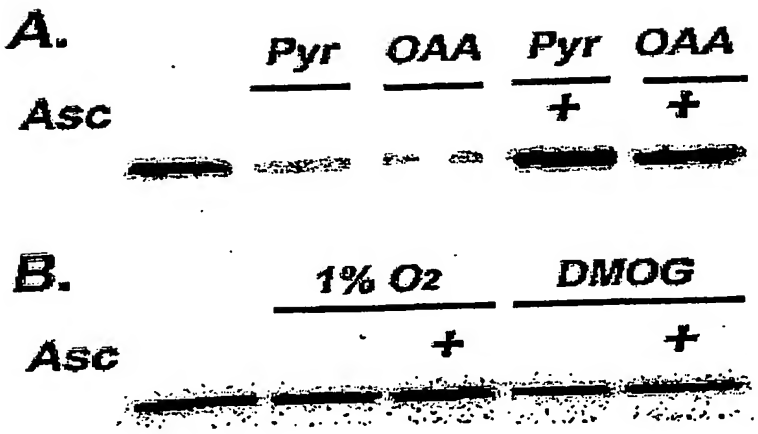


Figure 18

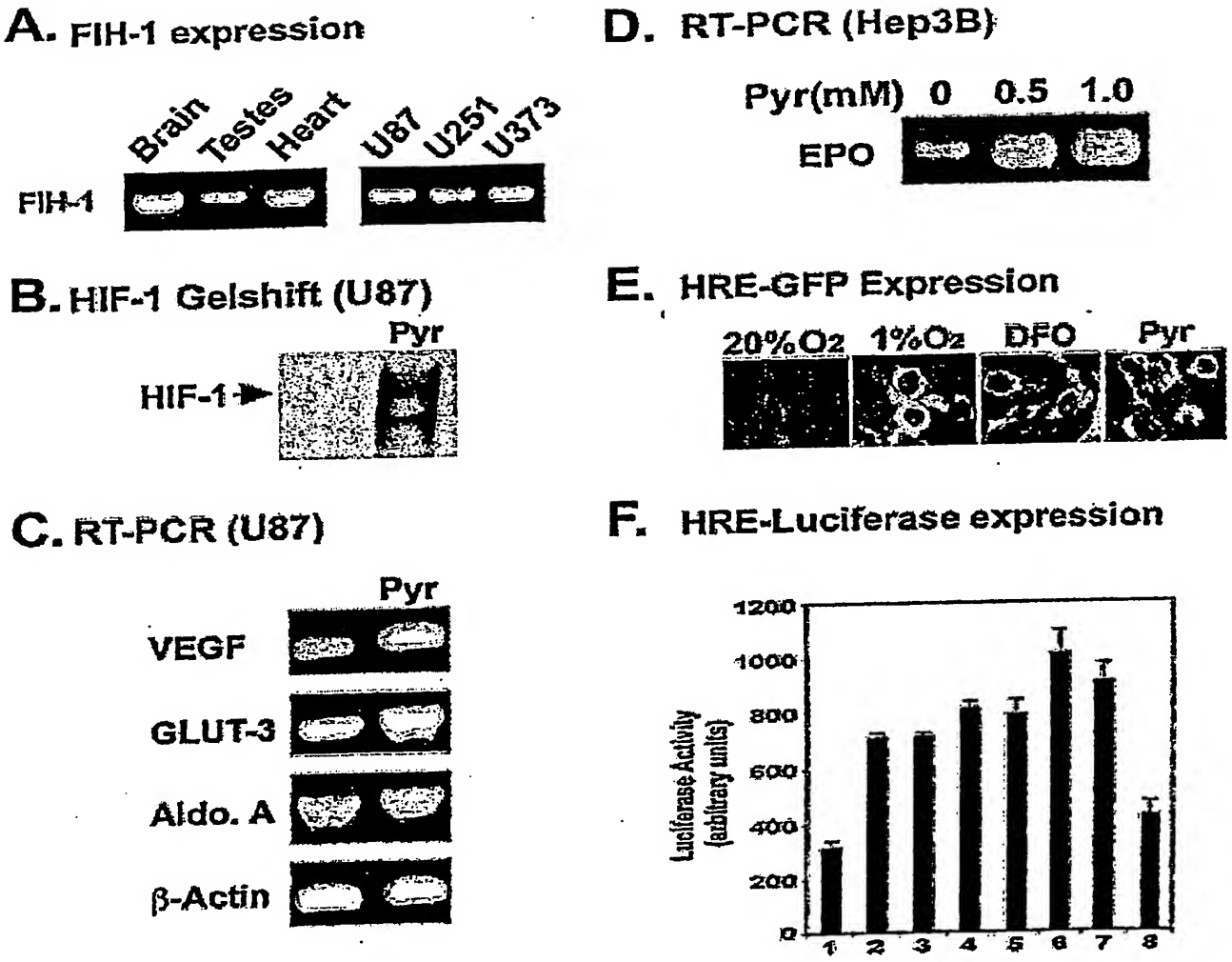

A. FIH-1 expression
Brain
Testes
Heart
U87
U251
U373
FIH-1
B. HIF-1 Gelshift (U87)
Pyr
HIF-1
C. RT-PCR (U87)
Pyr
VEGF
GLUT-3
Aldo. A
β-Actin
D. RT-PCR (Hep3B)
Pyr(mM) 0 0.5 1.0
EPO
E. HRE-GFP Expression
20%O2
1%O2
DFO
Pyr
F. HRE-Luciferase expression
Luciferase Activity (arbitrary units)
1200
1000
800
600
400
200
0
1 2 3 4 5 6 7 8

Figure 19

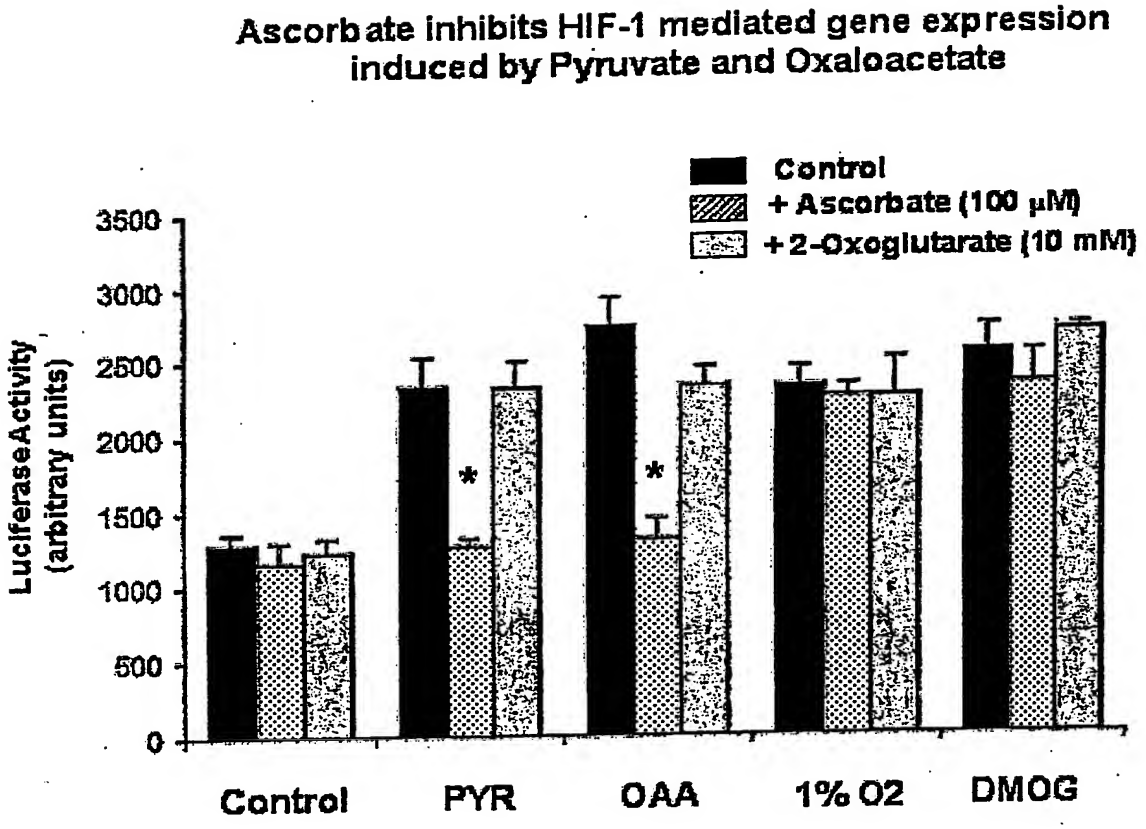

Ascorbate inhibits HIF-1 mediated gene expression induced by Pyruvate and Oxaloacetate
Control
+ Ascorbate (100 µM)
+ 2-Oxoglutarate (10 mM)
Luciferase Activity (arbitrary units)
3500
3000
2500
2000
1500
1000
500
0
*
*
Control
PYR
OAA
1% O2
DMOG

Figure 20

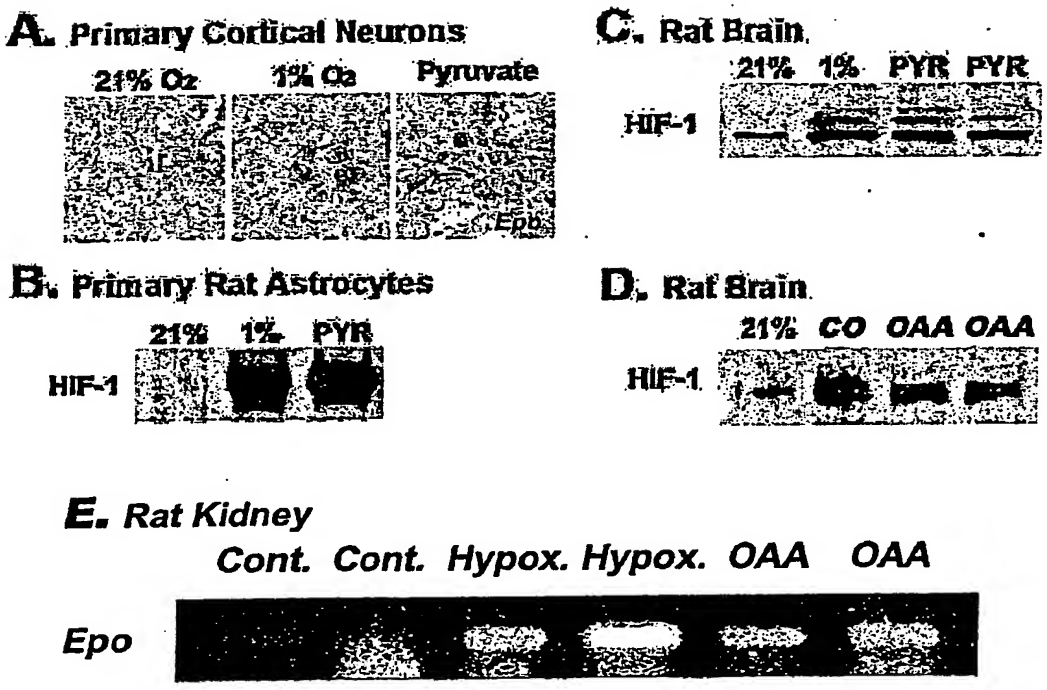
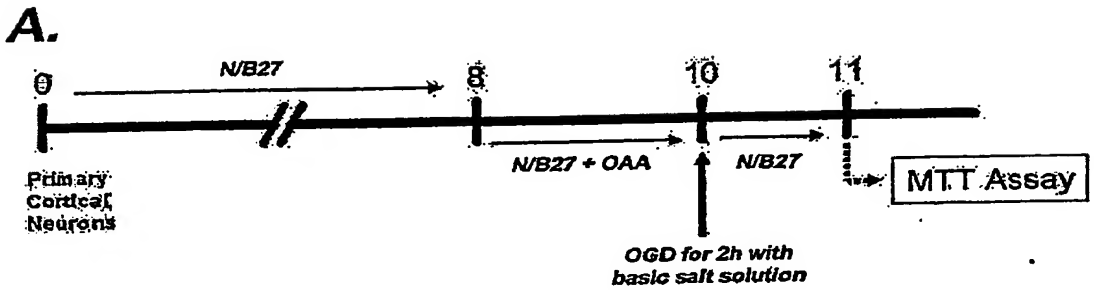
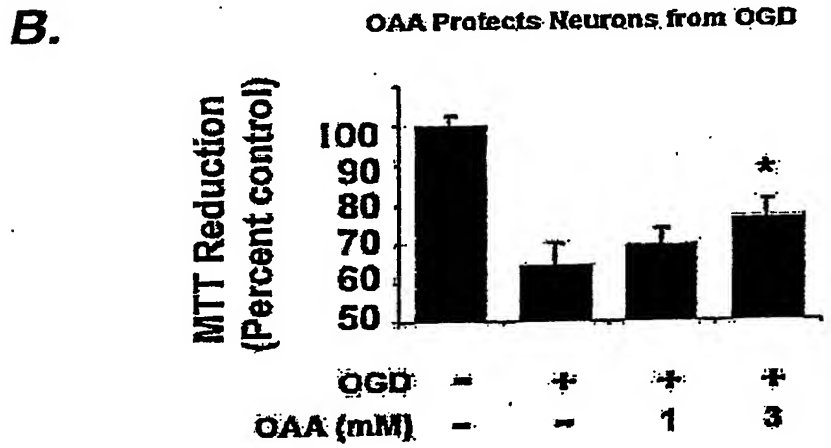

A. Primary Cortical Neurons
21% O2
1% O2
Pyruvate
Epo
B. Primary Rat Astrocytes
21%
1%
PYR
HIF-1
C. Rat Brain
21%
1%
PYR
PYR
HIF-1
D. Rat Brain
21%
CO
OAA
OAA
HIF-1
E. Rat Kidney
Cont.
Cont.
Hypox.
Hypox.
OAA
OAA
Epo

Figure 21


A.
0
N/B27
8
10
11
N/B27 + OAA
N/B27
MTT Assay
Primary Cortical Neurons
OGD for 2h with basic salt solution


B.
OAA Protects Neurons from OGD
MTT Reduction (Percent control)
100
90
80
70
60
50
OGD - + + +
OAA (mM) - - 1 3